

Inhibition of phosphoinositide 3-kinase γ attenuates inflammation, obesity, and cardiovascular risk factors

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Phosphoinositide 3-kinase γ (PI3K γ) plays a central role in inflammation, allergy, cardiovascular, and metabolic disease. Obesity is accompanied by chronic, low-grade inflammation. As PI3K γ plays a major role in leukocyte recruitment, targeting of PI3K γ has been considered to be a strategy for attenuating progression of obesity to insulin resistance and type 2 diabetes. Indeed, PI3K γ null mice are protected from high fat diet-induced obesity, metabolic inflammation, fatty liver, and insulin resistance. The lean phenotype of the PI3K γ -null mice has been linked to increased thermogenesis and energy expenditure. Surprisingly, the increase in fat mass and metabolic aberrations were not linked to PI3K γ activity in the hematopoietic compartment. Thermogenesis and oxygen consumption are modulated by PI3K γ lipid kinase-dependent and -independent signaling mechanisms. PI3K γ signaling controls metabolic and inflammatory stress, and may provide an entry point for therapeutic strategies in metabolic disease, inflammation, and cardiovascular disease.

Keywords: phosphoinositide 3-kinase; G protein-coupled receptors; chronic inflammation; obesity; atherosclerosis; leukocytes; adipocytes; thrombocytes; plaque formation

Introduction

Phosphoinositide 3-kinases (PI3Ks) have been shown to play key roles in the control of cellular metabolism, cell growth, proliferation, survival, and migration. The deregulation of these processes promotes chronic inflammation, tumor progression, and metabolic deviations (for a review, see Ref. 1). Class I PI3Ks produce the lipid second messenger phosphoinositol(3,4,5)-trisphosphate (PtdIns(3,4,5)P₃), which serves as a docking site for selected pleckstrin homology (PH) domain-containing proteins, including phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (PKB/Akt). While class IA PI3Ks are tightly associated with a p85-like regulatory subunit (encoded by the *PIK3R1*, *PIK3R2*, and *PIK3R3* gene loci) and are linked to protein tyrosine kinase receptor activation, the only member of the class IB PI3Ks, PI3K γ , forms a heterodimer with a p84 or p101 adaptor protein. PI3K γ operates downstream of G protein-coupled receptors (GPCRs)^{2,3} and is

activated by trimeric G protein G $\beta\gamma$ subunits. Both the p84 and p101 adaptor proteins sensitize the catalytic subunit of PI3K γ (p110 γ) for G $\beta\gamma$ input,⁴ but the p110 γ -p84 complex additionally requires interaction with the activated small G protein Ras to be fully operational.⁵ The p110 γ -p84 and p110 γ -p101 complexes can signal in a nonredundant fashion, and can operate in distinct plasma membrane micro-domains.³

PI3K γ promotes inflammation and modulates cardiovascular parameters

It was demonstrated early on that PI3K γ attenuates *in vivo* leukocyte migration toward chemokines: neutrophil recruitment² and dendritic cell movement⁶ are attenuated in mice lacking a functional p110 γ subunit. Protection of 110 γ null animals was shown to involve control of the replenishment of inflammatory cells to inflammation sites in inflammatory and allergic disease models. This observation was also observed following selective PI3K γ

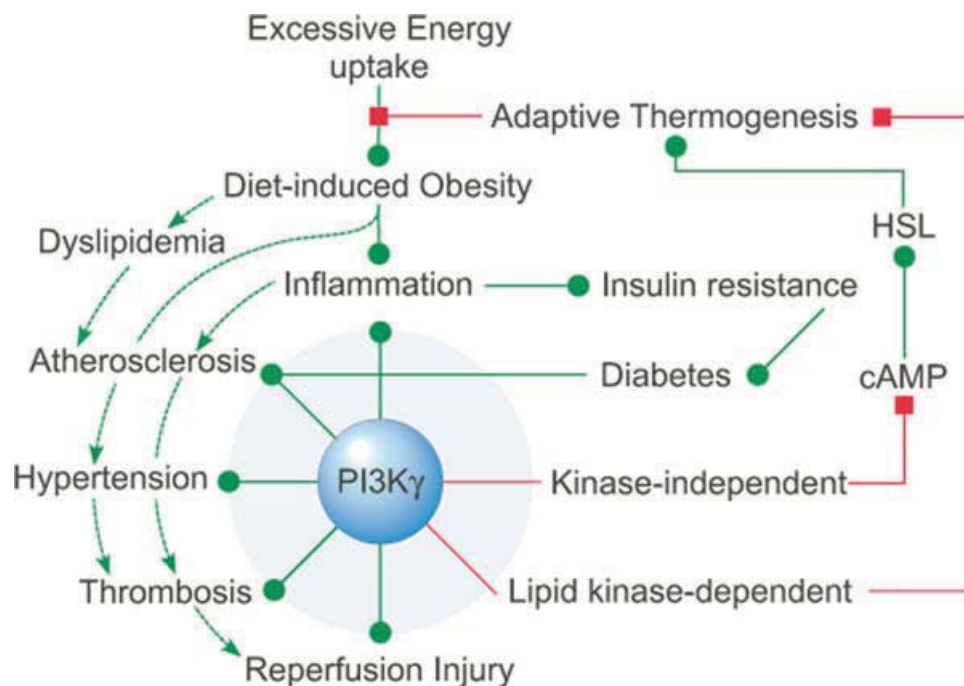


Figure 1. Implications of PI3K γ signaling in metabolic disease, obesity, and cardiovascular disease. PI3K γ downstream signaling attenuates adaptive thermogenesis via a kinase-independent and a lipid kinase-dependent pathway. Mice with functional PI3K γ therefore accumulate more body fat when kept on a high fat diet. The onset of obesity promotes the development of metabolic disease, resulting in dyslipidemia, metabolic inflammation, and insulin-resistant tissues. An obesity-dependent, persistent low-grade inflammatory state contributes to macrophage recruitment to adipose tissue. Furthermore, PI3K γ drives macrophage accumulation in the intima, and thus contributes to atherosclerotic plaque formation. Hypertension, linked to PI3K through the action of angiotensin II, favors rupture of atherosclerotic plaques. Finally, thrombosis can lead to blockage of coronary arteries. After a cardiac event, PI3K γ can support inflammatory processes worsening reperfusion injury. HSL: hormone sensitive lipase.

inhibition. It was subsequently demonstrated that inhibition of PI3K γ alleviated symptoms in rheumatoid arthritis⁷ and systemic lupus mouse models,⁸ and that anaphylaxis was promoted by mast cell activation and degranulation driven by a PI3K γ -dependent autocrine/paracrine activation loop.⁹

Furthermore, in PI3K γ -null platelets, aggregation and thrombosis were reduced in response to ADP. Loss of PI3K γ activity correlates with attenuated α (IIb) β (3) fibrinogen receptor activation and protects from ADP-induced platelet-dependent thromboembolic vascular occlusion.¹⁰ While cells of hematopoietic origin express high levels of p110 γ protein, PI3K γ also plays an important role in tissues where it is less prominently expressed. For example, in cardiomyocytes p110 γ is linked to cardiac contractility, serving as a scaffold protein for phosphodiesterase 3B (PDE3B). Lack of p110 γ leads to increased basal and adrenergic receptor-

induced cAMP-protein kinase A (PKA) signaling in cardiomyocytes, and this translates into increased heart contractile force. Mice expressing a catalytically inactive (KI) p110 γ (with Lys833 mutated to Arg), mimicking pharmaceutical inhibition of the enzyme, show no increase in cAMP, demonstrating that this pathway operates independently of PI3K γ lipid kinase activity. Moreover, p110^{KI/KI} mice were protected in a model of cardiac overload.¹¹

In atherosclerosis, the role of inflammation has attracted attention, as some steps in the action of atherogenic lipoproteins and cytokines act through PI3K. PI3K γ seems to promote early steps in the generation of atherosclerotic lesions. In murine models of atherosclerosis, such as the apolipoprotein E (ApoE)-null and low density lipoprotein receptor (LDLR)-null mice, either genetic (ApoE^{-/-} \times p110 γ ^{-/-} or LDLR^{-/-} \times p110 γ ^{-/-}) or pharmacological manipulation of PI3K γ activity

counteracted plaque formation. Interestingly, plaques forming in p110 γ -null mice were stabilized by the inclusion of increased levels of collagen, which clinically correlates with a better prognosis. Plaque formation was also linked to the hematopoietic cell lineages, as the transplantation of p110 γ ^{-/-} bone marrow into a wild-type host recapitulated the p110 γ -null phenotype. Mechanistically, loss of p110 γ decreases macrophage and T cell infiltration into the intima.^{12,13} Once the atherosclerotic lesions progress to narrowing of blood vessels through stenosis, smooth muscle migration has been reported to be potentiated by PI3K γ -dependent signals.¹⁴

As atherosclerotic plaques are formed, high blood pressure is a major risk factor for subsequent plaque rupture, stenosis, and, finally, fatal thrombotic events. In the development of hypertension, the renin-angiotensin system plays a major role by generating angiotensin II that elicits reactive oxygen species (ROS) within endothelial cells via GPCR stimulation. ROS production and the opening of PI3K γ -dependent L-type Ca²⁺ channels, leading to influx of extracellular Ca²⁺, mediate smooth muscle contraction. Most importantly, mice lacking PI3K γ are protected from angiotensin II-induced hypertension, ROS production, and vascular damage.¹⁵

PI3K γ -insulin resistance, control of thermogenesis, and obesity

Obesity is accompanied by chronic low-grade inflammation (metabolic inflammation), which has been proposed to be a cause of progressing insulin resistance, leading to the initiation of type II diabetes in obese patients.^{16,17} It was also proposed that metabolic inflammation affects energy balance during the development of obesity.¹⁸ Indeed, clinical studies suggest that specific anti-inflammatory treatments can improve glucose homeostasis in diabetics. Along these lines, we and others have recently found that loss of functional PI3K γ leads to a major improvement of insulin sensitivity in mice kept on a high fat diet. Obesity-dependent macrophage infiltration into adipose tissue was attenuated in p110 γ -null animals, and macrophage markers and inflammatory cytokine profiles were reduced in white adipose tissue.^{19,20} One interesting outcome of our study was the observation that p110 γ ^{-/-} mice on a high fat diet accumulated substantially less fat mass than wild-type mice, while

calorie intake and nonadipose tissue mass was unaffected. The difference in body weight increase could be linked to increased thermogenesis in p110 γ -null animals, triggered by lipid kinase-dependent and -independent pathways. Moreover, the lean phenotype accompanying increased thermogenesis in p110 γ -null mice was independent from PI3K γ activity within the hematopoietic compartment, since the energy expenditure and oxygen consumption was determined by the PI3K γ status of the host and not by the genotype of the transplanted bone marrow.²⁰

The PI3K γ signaling signature reflects a role in control of metabolic and inflammatory stress. As described above and summarized in Figure 1, PI3K γ inhibition might constitute the entry point for strategies for therapeutic intervention in metabolic and inflammatory disease. Additionally, multiple connections to PI3K γ signaling involve processes in atherosclerotic plaque formation, platelet aggregation, hypertension, and others, providing novel possibilities for cardioprotective therapies.

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Conflicts of interest

The authors declare no conflicts of interest.

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